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(54) Title: METHOD

(57) Abstract: There is described a method of magnetically manipulating a cell *in vivo* which comprises the association of a magnetisable particle with a cell. More particularly, there is described a method of magnetically manipulating a cell which comprises the association of a magnetisable particle with a cell characterised in that the method comprises agonising or antagonising ion channels within a cell by the association of a magnetisable particle with a cell. There is also described the use of a magnetisable particle in a method of magnetically manipulating a cell *in vivo* and/or activating ion channels *in vivo*.

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Method

This invention relates to a novel method of magnetically manipulating cells *in vivo* and to methods of treatment related thereto.

It is well established in pharmacology that communication, e.g. between cells, is governed by ion channels within the cells. A wide variety of such channels exist, for example potassium, calcium and sodium channels. Pharmaceutically active chemical compounds are often used to block such channels resulting in a pharmacological effect. For example, calcium antagonists are known to be active on the cardiovascular system, for example by reducing the magnitude of the calcium current in the sino-atrial and atrio ventricular nodes. An important aspect of ion channel control is determining when the channel opens (gating).

Ion channels generally possess ionic selectivity which is an extremely important aspect of the channel's functional properties. Channels are generally characterised by their ionic selectivity, for example

- sodium channel
- potassium channel
- calcium channel
- chloride channel
- non-selective cation channel.

Ion channels are large integral membrane proteins that form pores through a cellular plasma membrane allowing ions to cross by flowing down an electrochemical gradient through the channels (passive transport). The core of the pore is generally hydrophilic, and contains a part of the protein which recognises only certain ions thus acting as a selectivity filter. Gates in the channel can open in response to a variety of stimuli, including changes in membrane potential, mechanical activation or the presence of certain chemicals outside or inside the cell. More than 50 types of ion channels have been identified.

Ca channels, like Na channels, are voltage-gated, open when the internal voltage becomes more positive than the resting potential, and inactivate, or close, spontaneously even though the voltage stimulus is maintained. Ca channels are effective in the axon terminals of neurons, and in invertebrate muscle, vertebrate smooth muscle, and participate with Na channels in vertebrate cardiac muscle. Ca++ channels participate in action potentials when you need to get Ca++ into the cell to do something, such as make cardiac muscle contract, or release neurotransmitter at the axon terminal.

Na channels are almost all voltage-gated, that is, their gates open in response to changes in membrane potential, usually when the inside of the cell becomes more positive. Most Na channels are closed, or inactivate, spontaneously in a few milliseconds even though the membrane potential remains at the level which opened them. Na channels are found in neurons, vertebrate skeletal muscle, and cardiac muscle. Na serves to let charge into the cell; the Na itself doesn't do anything chemically.

Potassium channels, like Na channels, tend to be voltage-gated and to open when the inside of the cells becomes more positive. They mostly open at voltage more positive than Na or Ca channels, and most of them stay open as long as the voltage stays positive. Since the Nernst potential for K is near -80mV, opening K channels at voltages near +20mV lets K out and makes the internal voltage more negative. This in turn closes the K channels. This is how the action potential repolarises, or returns to resting potential. There are also K channels that are not voltage-gated. These are open at the resting potential, and in fact set the resting potential.

US Patent No. 6,548,264 describes silica coated nanoparticles which comprise a magnetic metal core. The magnetic core present in the particles enables the particles to be responsive to a magnetic field and therefore, the particles are suitable for use in diagnostic, imaging and recording systems. However, the nanoparticles of the prior

art may suffer from the disadvantage that they do not define the method of activation at a cellular level.

Magnetic bead twisting cytometry has been used to define the mechanical properties of single cells and to demonstrate that external mechanical forces can be transmitted across the cell surface and through the cytoskeleton via transmembrane cell adhesion molecules such as integrins, see, for example, Wang, N and Ingberger, DE (1995) Probing transmembrane mechanical coupling and cytomechanics using magnetic twisting cytometry. *Biochem. Cell Biol.* 73: 327-335.

We have now found a method of selectively activating cells which enables the cells to then be manipulated mechanically in a remote manner, e.g. from outside the body.

Thus according to the invention we provide a method of magnetically manipulating a cell *in vivo* which comprises the association of a magnetisable particle with a cell.

The method may comprise *ex vivo* manipulation of an *in vivo* process. Furthermore, it will be understood by the skilled man that a reference to a cell shall be construed to include a plurality of cells.

More particularly, the invention provides a method of agonising or antagonising ion channels within a cell which comprises the association of a magnetisable particle with a cell as hereinbefore described.

According to a further aspect of the invention we provide a method of magnetically manipulating a cell which comprises the association of a magnetisable particle with a cell characterised in that the method comprises agonising or antagonising ion channels within a cell by the association of a magnetisable particle with a cell.

In this aspect of the invention the magnetisable particle may be associated directly with the cell. Alternatively, the method may comprise associating the magnetisable

particle with an antibody, enzyme, etc., which is subsequently associated with the cell.

The association of a magnetisable particle with a cell may comprise the introduction of such a particle into a cell, the attachment of such a particle to a cell, e.g. externally or internally to a cell, or any combination thereof. Thus, the magnetisable particles may be associated intracellularly or extracellularly or a combination of intracellularly and extracellularly. However, in a preferred aspect of the invention the particles are associated intracellularly.

When the method of the invention comprises intracellular association this will comprise association with an internal binding site. By way of example only, for TREK-1, the particle(s) may be associated with the N-terminus region of the ion channel. Alternatively, the particle(s) may be associated with the COOH terminus region of the ion channel. It will be appreciated by one skilled in the art that numerous ion channels and binding sites may be utilised in the method of the invention. Thus, internal binding sites which correspond to the N-terminus region of the ion channel, as seen in TREK-1or which corresponds to the COOH terminus region of the ion channel. as seen in TREK-1 may be utilised as well as other binding sites known *per se*.

Thus, we also provide a method of manipulating a mechanosensitive ion channel characterised in that the method comprises the association of a magnetisable particle with an ion channel, either directly or indirectly.

The method of the invention may comprise the manipulation of mammalian cells or other cell types, such as bacterial cells, plant cells, etc. However, it will be understood by the skilled man that the method of the present invention may be used to manipulate other cell types not mentioned herein. Furthermore, the method may be an *in vitro* method or an *in vivo* method, although an *in vivo* method is preferred.

Preferentially, the method of the invention comprises the remote manipulation of cells and/or of agonising or autagonising ion channels, e.g. manipulation from outside the body., i.e. remote mechanical activation.

The method of the invention may be utilised in relation to a variety of cells which are known *per se*. However, preferentially, the method is suitable for use with mammalian somatic cells, for example, bone, cartilage, muscle (skeletal and cardiac) lymphatic cells,. endocrine cells, urinary system cells, cells relating to the reproduction system, neuronal cells and tumour cells.

The method of the invention may be utilised in connection with any conventionally known ion channels within the cell which are hereinbefore described. The method is especially suited for use in mechanosensitive ion channels. Such mechanosensitive ion channels have been identified in many cell types and have been predominantly described as calcium or potassium ion channels, although it should be understood that the method of the invention is not limited to use in relation to calcium or potassium ion channels. By way of example only, one such channel which has been well characterised at the molecular level and at the functional level in neuronal cells is the chromosomal gene TREK-1, which is part of the 2P K+ channel family. TREK-1 channels, have been identified in bone cells, and are known to respond to shear stress, cell swelling and membrane stretch as well as other external agents such as fatty acids and general anaesthetics.

A particular aspect of the present invention is to provide a method of manipulating mechanosensitive ion channels.

These "mechanosensitive" ion channels are present in a variety of mammalian, e.g. human, and bacterial cells and the present invention enables the cells to be selectively activated in the body and/or in cell cultures, see, for example, Sokabe, M, F Sachs, A Jing (1991) Quantitative video microscopy of patch clamped membranes: Stress, strain, capacitance, and stretch channel activation. *Biophys J.* 59: 722-728; Stewart,

Z, B Martinac and J Dobson (2000) Evidence for mechanosensitive transmembrane ion channels of small conductance in magnetotactic bacteria. *Electro- and Magnetobiol.* **19**: 81-89. As these channels are instrumental in normal cellular function and play a particularly important role in, for example, the production of bone and connective tissue or activation of the peripheral nervous system, the ability to manipulate them remotely, e.g. from outside the body, is especially advantageous an provides applications in, *inter alia*, pain relief, e.g. anaesthetics, therapeutics, tissue engineering and repair and cancer therapy.

In a further aspect of the invention the method may also be suitable for use with conventionally non mechanosensitive cells and/or ion channels by the transfection of channels into cells which may otherwise be otherwise non-responsive.

All ion channels open and close (i.e. change conformational state) in response to forces and this is the principle behind ion channel activation. In the case of mechanosensitive ion channels, the force results in membrane deformation, triggering the opening of the channel. Voltage-gated and ligand-gated ion channels are also "mechanoresponsive" in that they respond to mechanical stresses on the ion channel generated by coulomb forces (in the case of voltage-gated channels) and binding forces (in the case of ligand-gated channels). As such, all ion channels can be activated by the method described herein provided that the magnetisable particle is coupled, either directly or indirectly, to the mechanoresponsive region of the channel protein.

Thus, in one aspect of the present invention the ion channel is a voltage-gated ion channel, alternatively, the ion channel is a ligand-gated ion channel.

A wide variety of particles may be used in the method of the invention. The magnetisable particle used in the method of the invention may be inherently magnetic or, alternatively, may be one which reacts in a magnetic field. Generally, any magnetic material may be used, however, by the term magnetic we mean, for

example, a material which is paramagnetic superparamagnetic, ferromagnetic and/or antiferromagnetic, examples of which include elemental iron (Fe), or an compound, e.g. an iron salt, such as, magnetite (Fe_3O_4), maghemite (γFe_2O_3), and greigite (Fe_3S_4), or a chromium compound, e.g. a chromium salt, such as chromium oxide (CrO_2), or any combination thereof. Preferably the magnetic material comprises particles, e.g. nanoparticles, which comprises a magnetic core with a biocompatible coating. Thus, such preferred particles are nanoparticles and especially nanoparticles having a core and, e.g. a silica shell enveloping the core. However, also porous particles with multiple magnetic centres within the pores. An example of such particles are those nanoparticles described in US Patent No. 6,548,264 which is incorporated herein by reference. Thus, the prior art nanoparticles may have a mean size of less than 1 micron, each of said nanoparticles comprising (a) a core comprising a magnetisable particle and (b) a silica shell enveloping the core, wherein the magnetisable particle is a magnetic material as hereinbefore described.

The micro- and nano- particles (intended to be attached to the cells) will generally be substantially spherical or elliptical. The size of the particles may vary according, *inter alia*, to the nature of the magnetisable material, the application, etc. However, an example of particles may be nanoparticles can having a mean size, e.g. diameter, of 5000 nm or less, e.g. from 1 nm to 5000 nm, preferably from 1 nm to 1000 nm, more preferably from 1 nm to 300 nm, or from 2 nm to 10 nm).

The particles for attachment to the cells may be coated or uncoated and single or multi-domain. Examples of suitable particles include, but are not limited to:

(i) Coated magnetic microspheres (d = 4 μm) available from Spherotech, Inc. These microspheres consist of a magnetically blocked core - coated by a polymer.

(ii) Single-domain, ferrite-doped silica nanoparticles with tunable size (d = 50-300 nm) and narrow size distribution.

In the method of the invention the ion channels may be activated by attaching the magnetisable particles as hereinbefore described to specific regions of the cellular membrane and/or to specific "receptors". on the ion channels themselves. Thus, the mechanical forces required to activate the channels can then be applied remotely by a magnetic field acting on these magnetic particles.

In particular the method of the invention comprises modifying a magnetisable particle as hereinbefore described by tagging the particle with one or more specific antibodies or protein binding motifs which recognise key cellular elements within a cell. These include transmembrane adhesion molecules, such as integrins, cadherins, selectins, and immunoglobulins or dispersed membrane adhesion proteins such as RGD (arginine-glycine-aspartate), see, for example, . J. Chen, B. Fabry, E. L. Schiffrin, and N. Wang (2001) Twisting integrin receptors increases endothelin-1 gene expression in endothelial cells *Am J Physiol Cell Physiol.* **280**: 1475-84 ; A. R. Bausch, U. Hellerer, M. Essler, M. Aepfelbacher, and E. Sackmann (2001) Rapid stiffening of integrin receptor-actin linkages in endothelial cells stimulated with thrombin: a magnetic bead microrheology study *Biophys J* **80**: 2649-57 ; Cartmell, SH, J Dobson, S Verschueren, A El Haj (2002) Development of magnetic particle techniques for long-term culture of bone cells with intermittent mechanical activation. *IEEE Transactions on NanoBioscience* **1**: 92-97.

The method of the invention is especially advantageous because it provides a method of treatment of a variety of disorders. Indeed the invention provides a method of treatment which is applicable to any disorder in which one or more ion channels play a role. In addition, the invention provides a method for potential control of ion channel activation including pain relief, e.g. an anaesthetic role.

Thus according to the invention we provide a method of treatment of a patient suffering from a disorder in which an ion channel plays a role which comprises the

administration to such a patient of magnetisable nanoparticles as hereinbefore described and manipulating those particles using a magnetic field.

The method of treatment as hereinbefore described should not be considered to be limited, but it is especially advantageous in tissue and/or bone repair. The method of treatment can be to facilitate further treatment by providing a method of pain relief, e.g. for localised anaesthesia, to targeted regions of the body.

The nature of such cells may vary depending upon the nature of the tissue of interest. For example, the cells may be ligamentum cells for growing new ligaments, tenocytes for growing new tendon. Alternatively, the cells may be chondrocytes and/or other stromal cells, such as chondrocyte progenitor cells.

Thus the method of the invention may include the regeneration of tissue or the generation of artificial tissue, such as skin, cartilage, ligament, tendon, muscle or bone.

Alternatively the method may comprise wound healing and/or tissue adhesion.

In a preferred embodiment the method may comprise bone repair and/or bone growth.

In a yet further alternative the method of the invention may include, for example, dental applications and/or veterinary applications.

The method also may be used as a mechanism for selectively killing cells (such as tumour cells) *in vivo*. In this case, magnetisable particles are attached to the target cell membrane or ion channel protein and a magnetic field is applied to the *in vivo* target region. The rapid, cyclic opening and closing (via the application of a time varying magnetic field), and/or the holding open (via the application of a static

magnetic field) of ion channels in the cell membrane allows ions (such as Ca^{++}) to flood the cell, inducing osmotic shock and, consequently, cell death.

Thus, according to this aspect of the invention we also provide a method of destroying cells or inhibiting cell growth which comprises agonising or antagonising ion channels within a cell which by the association of a magnetisable particle with a cell.

The method may comprise a method of inducing osmotic shock to a cell, e.g. by agonising or antagonising ion channels within a cell by the association of a magnetisable particle with a cell. The method is especially useful in the treatment or alleviation of a tumour cell, e.g. a cancer cell.

Thus, the method may comprise the killing of cells by holding ion channels open with a targeted static magnetic field. Alternatively, the method may comprise the killing of cells via cyclically opening and closing ion channels with a targeted, time-varying magnetic field.

In the methods of the invention the magnetic field may be varied depending upon, *inter alia*, the nature of the disorder to be treated, but may be, for example, at a frequency of from 0.1 to 10 Hz. But, frequencies outside this range can also be used. The magnetic field will typically have a flux density in the order of (but not limited to) 10 mT to 1400 mT.

In the method of the invention the magnetic field may be generated outside the body for the case of *in vivo* applications, and may be provided by a permanent magnet or an electromagnet. The magnetic field may be a constant or a variable field, e.g. a permanent magnet may be moved relative to the cells. In the case of an electromagnet, a magnetic field may be generated by provision of appropriate electric current levels to the electromagnetic, optionally, in combination with alternating current.

According to a yet further aspect of the invention we provide a method of inducing a therapeutic effect in a cell which comprises agonising or antagonising ion channels within the cell by the association of a magnetisable particle with the cell and magnetically manipulating the magnetisable particle.

In addition we provide a method of treatment which comprises the administration of a therapeutically active agent which may be administered simultaneously, separately or sequentially with a magnetisable particle whilst agonising or antagonising ion channels within the cell.

We also provide a method of targeting a therapeutically active agent to a cell which comprises agonising or antagonising ion channels within the cell by the association of a magnetisable particle with the cell, magnetically manipulating the magnetisable particle and simultaneously, separately or sequentially administering the therapeutically active agent.

According to a yet further aspect of the invention we also provide the use of a magnetisable particle in a method of magnetically manipulating cells *in vivo*

The use may comprise *ex vivo* manipulation of an *in vivo* process. More particularly, the invention provides the use of a magnetisable particle in the manufacture of a system for magnetically manipulating a cell which system comprises the association of a magnetisable particle with a cell and agonising or antagonising ion channels within the cell.

In this aspect of the invention the magnetisable particle may be associated directly with the cell. Alternatively, the use may comprise associating the magnetisable particle with an antibody, enzyme, etc., which is subsequently associated with the cell.

When the use of the invention comprises intracellular association. By way of example only, for TREK-1, the particle(s) may be associated with the N-terminus region of the ion channel. Alternatively, the particle(s) may be associated with the COOH terminus region of the ion channel.

The use of the invention may comprise the manipulation of mammalian cells or other cell types, such as bacterial cells, plant cells, etc. The use may be an *in vitro* use or an *in vivo* use, although an *in vivo* use is preferred.

Preferentially, the use of the invention comprises the remote manipulation of cells and/or of agonising or autagonising ion channels, e.g. manipulation from outside the body, i.e. remote mechanical activation.

The use of the invention may be utilised in relation to a variety of cells, which are known *per se*. However, preferentially, the use is suitable for use with mammalian somatic cells, for example, bone, cartilage, muscle (skeletal and cardiac) lymphatic cells, endocrine cells, urinary system cells, cells relating to the reproduction system, neuronal cells and tumour cells.

The use of the invention may be utilised in connection with any conventionally known ion channels within the cell, which is hereinbefore described. The use is especially suited for use in mechanosensitive ion channels hereinbefore described.

A particular aspect of the present invention is to provide the use in the manufacture of a system for manipulating mechanosensitive ion channels.

In a further aspect of the invention the use may also be suitable for use with conventionally non mechanosensitive cells and/or ion channels by the transfection of channels into cells which may otherwise be otherwise non-responsive.

In one aspect of the present invention the ion channel is a voltage-gated ion channel, alternatively, the ion channel is a ligand-gated ion channel.

A wide variety of particles may be used in the use of the invention. Generally, any magnetisable material may be used, examples of which include elemental iron (Fe), or an iron compound, e.g. an iron salt, such as, magnetite (Fe_3O_4), maghemite (γFe_2O_3), and greigite (Fe_3S_4), or a chromium compound, e.g. a chromium salt, such as, chromium oxide (CrO_2), or any combination thereof. Preferably the magnetic material comprises particles which comprises a magnetic core with a biocompatible coating. Thus, such preferred particles are nanoparticles and especially nanoparticles having a core and, e.g. a silica shell enveloping the core. However, also porous particles with multiple magnetic centres within the pores. An example of such particles are those nanoparticles described in US Patent No. 6,548,264 which is incorporated herein by reference.

In particular the use of the invention comprises modifying a magnetisable particle as hereinbefore described by tagging the particle with one or more specific antibodies or protein binding motifs which recognise key cellular elements within a cell. These include transmembrane adhesion molecules, such as integrins, cadherins, selectins, and immunoglobulins or dispersed membrane adhesion proteins such as RGD (arginine-glycine-aspartate).

The use of the invention is especially advantageous because it provides a system suitable for use in the treatment of a variety of disorders. Indeed the invention provides the use in the manufacture of a medicament suitable for a treatment, which is applicable to any disorder in which one or more ion channels play a role. In addition, the invention provides the use for potential control of ion channel activation including pain relief, e.g. an anaesthetic role.

Thus, according to the invention we provide the use of a magnetisable particle in the manufacture of a medicament suitable for the treatment of a patient suffering from a

disorder in which an ion channel plays a role which comprises the administration to such a patient of magnetisable particles as hereinbefore described and manipulating those particles using a magnetic field.

The use as hereinbefore described should not be considered to be limited, but it is especially advantageous in tissue and/or bone repair. The use can be to facilitate further treatment by providing a method of pain relief, e.g. for localised anaesthesia, to targeted regions of the body.

The nature of such cells may vary depending upon the nature of the tissue of interest. For example, the cells may be ligamentum cells for growing new ligaments, tenocytes for growing new tendon. Alternatively, the cells may be chondrocytes and/or other stromal cells, such as chondrocyte progenitor cells.

Thus, the use may include the regeneration of tissue or the generation of artificial tissue, such as skin, cartilage, ligament, tendon, muscle or bone.

Alternatively the use may comprise wound healing and/or tissue adhesion.

In a preferred embodiment the use may comprise bone repair and/or bone growth.

In a yet further alternative the use of the invention may include, for example, dental applications and/or veterinary applications.

The use also may be used as a mechanism for selectively killing cells (such as tumour cells) *in vivo* as hereinbefore described.

Thus, according to this aspect of the invention we also provide the use of a magnetisable particle in the manufacture of a system for destroying cells or inhibiting cell growth which comprises agonising or antagonising ion channels within a cell which by the association of a magnetisable particle with a cell.

The use may comprise use in a method of inducing osmotic shock to a cell, e.g. by agonising or antagonising ion channels within a cell by the association of a magnetisable particle with a cell. The use in this aspect of the invention is especially useful in the treatment or alleviation of a tumour cell, e.g. a cancer cell.

Thus, the use may comprise the killing of cells by holding ion channels open with a targeted static magnetic field. Alternatively, the use may comprise the killing of cells via cyclically opening and closing ion channels with a targeted, time-varying magnetic field.

According to a yet further aspect of the invention we provide the use of a magnetisable particle in the manufacture of a system for inducing a therapeutic effect in a cell which comprises agonising or antagonising ion channels within the cell by the association of a magnetisable particle with the cell and magnetically manipulating the magnetisable particle.

In addition we provide the use of a magnetisable particle in the manufacture of a system comprising a therapeutically active agent which may be administered simultaneously, separately or sequentially with the magnetisable particle whilst agonising or antagonising ion channels within the cell.

We also provide the use of a magnetisable particle in the manufacture of a system for targeting a therapeutically active agent to a cell which comprises agonising or antagonising ion channels within the cell by the association of a magnetisable particle with the cell, magnetically manipulating the magnetisable particle and simultaneously, separately or sequentially administering the therapeutically active agent.

According to a yet further aspect of the invention we provide a kit comprising a therapeutically active agent and means for associating a magnetisable particle with a cell.

It will be understood by the skilled that any conventionally known therapeutically active agent or a combination of therapeutically active agents may be utilised in the kit of the invention.

Thus, the kit may comprise a vessel containing a therapeutically active agent, a source of magnetisable particles and instructions for the simultaneous, sequential or separate administration thereof. The kit of the invention may also include other agents known *per se*. The invention may also include the use of a kit as hereinbefore described in the manufacture of a medicament.

The invention will now be described by way of example only and with reference to the accompanying drawings in which Figure 1a) is a schematic representation of the structure of TREK-1 showing the three sites of 12x histidine insertions for tagging magnetic beads for mechanical manipulation;
Figure 1b) illustrates primary human astrocytes with membrane bound RGD coated carboxyl ferromagnetic particles (4μm) (magnification x 1000);
Figure 2 is a schematic of the TREK ion channel showing structure and location of the His. tags present in the protein. Red circles indicate the sites of the His tags at the three sites, the primary loop, the COOH terminus and the NH terminus;
Figure 3 is a representation of the magnetic activation of Trek-1 monitored via downstream changes in intracellular calcium; and
Figure 4 is a representation of the magnetic activation of TREK-1 induces transient rise in intracellular calcium in HEK293 T cells co-transfected with and Flashpericam.

Example 1

Activation of TREK-1 using magnetic cytometry

The modified TREK-1 gene was transvected into the human HEK 293 cell line. Detection of plasmid transfection efficiency was conducted by monitoring CD8

expression using immunocytochemsitry, electrophysiology using whole cell recordings and a fluorescent marker for membrane depolarisation monitored via confocal microscopy. Three regions of the molecule were tagged for experimental manipulation by insertion for a 12x histidine coding sequence as shown in Figure 1a. Functionalised, magnetic micro- and nano-particles were coupled to the cell membrane using the 12x His antibody coatings to enable force to be applied to different regions of the channel. Particles were twisted by moving high-field rare earth magnets (NdFeB) magnets with surface flux density of up to 1.4 Tesla, about a vertical axis near the cell culture. During this process, cells were monitored via phase contrast microscopy and confocal microscopy. Electrical activity in cells were monitored using whole cell recordings.

Specific monoclonal antibodies raised to the three regions prior to histidine insertions outlined above have been raised for tagging endogenous TREK channels in vivo.

Example 2

Non-specific membrane deformation using magnetic cytometry

Biocompatible magnetic micro- and nanoparticles were coupled to the cell membrane (specifically with monoclonal antibodies to RGD containing peptides and collagen) to stretch generalised regions of the cell (Figure 1b). The torque applied to magnetically blocked particles deforms the cell membrane and activates nearby MS ion channels following application of a range of magnetic fields. In addition, the cells were biochemically assayed to determine whether reaction pathways are being initiated by magnetic twisting (e.g. prostaglandin and extracellular matrix production). MS ion channel blockers such as gadolinium or amiloride were added extracellularly to confirm whether the MS channels are instrumental in any observed changes.

Initial experiments employed functionalised magnetic microspheres (d=4 μm) available from Spherotech. Inc. In addition magnetically blocked, ferrite-doped silica nanoparticles with tunable size (d=50-300nm) and narrow size distribution and

PVA/magnetite nanoparticle-based ferrofluids (d=4-10nm) were synthesised. High-field rare earth magnets again were used to generate the applied fields.

Example 3

Magnetic activation in a 3D model

The use of magnetic strategies for spatially targeted ion channel activation in a 3D, cell-seeded scaffold was investigated by applying a magnetic field across a cell-seeded construct within a bioreactor. The ion channels in the cells were activated within the scaffold and the long-term effects of this ion channel activation on matrix synthesis and cell proliferation assessed. Magnetic particle-based approaches with a non-specific activation and a TREK-transfected bone and cartilage cell lined model were used.

Example 4

Calcium channel activity following attachment of magnetic particles to anti-His antibody

COS-7 cells were co-transfected with FLASH-pericam and 6His. TREK-1. 1μm anti-His antibody coated super-paramagnetic particles were then attached to the surfaces of transfected cells and manipulated by the application of a magnetic field (750G). Flash pericam was used to monitor changes in intracellular calcium activity following the magnetic activation and subsequent exposure to 100μm Riluzole, a chemical activator of TREK-1.

Referring to Figure 3, COS-7 cells were co-transfected with 'Flash' pericam and 12His.Trek-1 – Red and yellow lines indicate the cells with magnetic particles attached. Green and White lines indicate the cells without magnetic particles attached. The Magnetic field applied for 1 sec – yellow arrow

Application of 100uM Riluzole to the culture media – red arrow.

Referring to Figure 4, Channel 1= Green = HEK293 cells with magnetic particle attached = white arrow indicates calcium response, Channel 2 = demonstrates

movement of the particles attached to the cells following application of the magnet (indicated by a red arrow).

Claims

1. A method of magnetically manipulating a cell *in vivo* which comprises the association of a magnetisable particle with a cell.

2. A method of magnetically manipulating a cell which comprises the association of a magnetisable particle with a cell characterised in that the method comprises agonising or antagonising ion channels within the cell .

3. A method according to claims 1 or 2 characterised in that the method comprises associating the magnetisable particle with an antibody, or an enzyme.

4. A method according to claim 1 or 2 characterised in that particles are associated intracellularly or extracellularly.

5. A method according to claim 4 characterised in that particles are associated intracellularly.

6. A method according to claim 5 characterised in that particles are associated with the N-terminal region of the ion channel.

7. A method according to claim 5 characterised in that particles are associated with the COOH terminal region of the ion channel.

8. A method according to claim 2 characterised in that the method is an *in vivo* method.

9. A method according to claim 2 characterised in that the method is an *ex vivo* method.

10. A method according to claims 1 or 2 characterised in that the particle is a nanoparticle.

11. A method according to claims 1 or 2 characterised in that the method comprises the remote manipulation of a cell.

12. A method according to claims 1 or 2 characterised in that the cell is a mammalian cell.

13. A method according to claims 1 or 2 characterised in that the cell is a bacterial cell.

14. A method according to claims 1 or 2 characterised in that the cell is a plant cell.

15. A method according to claim 11 characterised in that the cell is derived from connective or neuronal tissue.

16. A method according to claim 15 characterised in that the cell is derived from bone, neurons, cardiac cells or any combination thereof.

17. A method according to claim 2 characterised in that the ion channel is a mechanosensitive ion channel.

18. A method according to claim 17 characterised in that the mechanosensitive ion channel has been transfected into a cell.

19. A method according to claims 17 or 18 characterised in that the ion channel is a voltage-gated ion channel.

20. A method according to claims 17 or 18 characterised in that the ion channel is a ligand-gated ion channel.

21. A method according to claim 2 characterised in that the ion channel is selected from the group a including sodium channel, potassium channel, calcium channel, chloride channel and a non-selective cation channel or any combination thereof.

22. A method according to claim 21 characterised in that the ion channel is selected from a calcium or a potassium ion channel.

23. A method according to claim 22 characterised in that the ion channel is a potassium ion channel.

24. A method according to claim 23 characterised in that the potassium channel is a TREK-1 channel.

25. A method of manipulating a mechanosensitive ion channel characterised in that the method comprises the association of a magnetisable particle with an ion channel.

26. A method according to claim 1, 2 or 25 characterised in that the magnetisable material is selected from the group which includes elemental iron (Fe), or a compound thereof, and a chromium compound, or a combination thereof.

27. A method according to claim 26 characterised in that the iron compound is an iron salt.

28. A method according to claim 27 characterised in that the iron salt is selected from the group which includes magnetite (Fe_3O_4), maghemite (γFe_2O_3) and greigite (Fe_3S_4), or any combination thereof.

29. A method according to claim 26 characterised in that the chromium compound is a chromium salt.

30. A method according to claim 29 characterised in that the chromium salt is chromium oxide (CrO_2).

31. A method according to claim 1, 2 or 25 characterised in that the magnetic material comprises particles which comprises a magnetic core with a biocompatible coating.

32. A method according to claim 31 characterised in that the particle has a core and a silica shell enveloping the core.

33. A method according to claim 32 characterised in that the particle is selected from those comprising (a) a core comprising a magnetisable particle and (b) a silica shell enveloping the core.

34. A method according to claim 33 characterised in that the magnetisable particle is selected from the group, which includes elemental iron (Fe), or a salt thereof and a chromium salt, or a combination thereof.

35. A method according to claim 25 characterised in that the particle is a porous particle with multiple magnetic centre within the pores.

36. A method according to claim 1, 2 or 25 characterised in that the particles have a mean size of 5000 nm or less.

37. A method according to claim 36 characterised in that the particles have a mean size of from 1 nm to 5000 nm.

38. A method according to claim 1, 2 or 25 characterised in that the method comprises the application of a remote magnetic field on the magnetisable particles.

39. A method according to claim 1, 2 or 25 characterised in that the particle is tagged with one or more specific antibodies or protein binding motifs which recognise key cellular elements within a cell.

40. A method according to claim 37 characterised in that the specific antibodies or protein binding motifs are selected from transmembrane extracellular matrix molecules, adhesion molecules or dispersed membrane adhesion proteins or extracellular matrix proteins.

41. A method according to claim 40 characterised in that the method is *in vivo*.

42. A method according to claim 40 characterised in that the specific antibodies or protein binding motifs are transmembrane adhesion or extracellular matrix molecules.

43. A method according to claim 42 characterised in that the transmembrane adhesion molecules are selected from integrins, cadherins, selectins, and immunoglobulins.

44. A method according to claim 41 characterised in that the specific antibodies or protein binding motifs are selected from dispersed membrane adhesion proteins.

45. A method according to claim 44 characterised in that the dispersed membrane adhesion protein is RGD (arginine-glycine-aspartate).

46. A method of treatment of a patient suffering from a disorder in which an ion channel plays a role which comprises the administration to such a patient of

magnetisable particles as hereinbefore described and manipulating the ion channels or cells using a magnetic field external to the body.

47. A method of destroying cells or inhibiting cell growth which comprises agonising or antagonising ion channels within a cell by the association of a magnetisable particle with a cell.

48. A method of inducing osmotic shock to a cell which comprises agonising or antagonising ion channels within a cell by the association of a magnetisable particle with a cell.

49. A method of treatment or alleviation of a tumour cell which comprises a method according to claim 46.

50. A method according to claim 49 characterised in that the tumour cell is a cancer cell.

51. A method of treatment of a patient according to claim 47 characterised in that the method comprises the killing of cells via holding ion channels open with a targeted static magnetic field.

52. A method of treatment of a patient according to claim 47 characterised in that the method comprises the killing of cells via cyclically opening and closing ion channels with a targeted, time-varying magnetic field.

53. A method of treatment according to claim 47 in which a disorder may involve a number of tissues in the body where ion channels play a key role in normal cellular homeostasis.

54. A method according to claim 53 characterised in the cells are cardiac muscle cells.

55. A method according to claim 53 characterised in that the method comprises the treatment of hypertension.

56. A method according to claim 53 characterised in that the method comprises pain relief.

57. A method according to claim 56 characterised in that the method comprises anaesthesia.

58. A method according to claim 57 characterised in that the anaesthesia is localised.

59. A method of treatment of a patient according to claim 46 characterised in that the method comprises tissue and/or bone repair.

60. A method of treatment according to claim 59 characterised in that the cells are selected from ligamentum cells, tenocytes, chondrocytes and other stromal cells (such as chondrocyte progenitor cells).

61. A method of treatment according to claim 59 characterised in that the method comprises the regeneration of tissue or the generation of artificial tissue, such as skin, cartilage, ligament, tendon, muscle or bone.

62. A method of treatment according to claim 59 characterised in that the method comprises the remote activation of ion channels.

63. A method of treatment according to claim 59 characterised in that the method comprises wound healing and/or tissue adhesion.

64. A method of treatment according to claim 59 characterised in that the method comprises bone repair and/or bone growth.

65. A method of treatment according to claim 46 characterised in that the method comprises a dental or veterinary application.

66. A method for establishing localised anaesthesia through the action of ion channel modulation by a magnetic field external to the body.

67. A method according to claim 66 characterised in that the pain relief comprises anaesthesia.

68. A method of treatment according to claim 46 characterised in that the method comprises the use of a magnetic field at a frequency of from 0.1 to 10 Hz.

69. A method of treatment according to claim 46 characterised in that the method comprises the use of a magnetic field will typically have a flux density of from 10 mT to 1400 mT.

70. A method of inducing a therapeutic effect in a cell which comprises agonising or antagonising ion channels within the cell by the association of a magnetisable particle with the cell and magnetically manipulating the magnetisable particle..

71. A method of treatment which comprises the administration of a therapeutically active agent which may be administered simultaneously, separately or sequentially with a magnetisable particle whilst agonising or antagonising ion channels within the cell.

72. A method of targeting a therapeutically active agent to a cell which comprises agonising or antagonising ion channels within the cell by the association of a magnetisable particle with the cell, magnetically manipulating the magnetisable

particle and simultaneously, separately or sequentially administering the therapeutically active agent.

73. The use of a magnetisable particle in a method of magnetically manipulating a cell *in vivo* wherein the method comprises the association of a magnetisable particle with a cell.

74. The use of a magnetisable particle in the manufacture of a system for magnetically manipulating a cell which system comprises the association of a magnetisable particle with a cell and agonising or antagonising ion channels within the cell.

75. The use according to claims 73 or 74 characterised in that the method comprises associating the magnetisable particle with an antibody, or an enzyme.

76. The use according to claim 73 or 74 characterised in that particles are associated intracellularly or extracellularly.

77. The use according to claim 76 characterised in that particles are associated intracellularly.

78. The use according to claim 77 characterised in that particles are associated with the N-terminal region of the ion channel.

79. The use according to claim 77 characterised in that particles are associated with the COOH terminal region of the ion channel.

80. The use according to claim 74 characterised in that the method is an *in vivo* method.

81. The use according to claim 74 characterised in that the method is an *ex vivo* method.

82. The use according to claims 73 or 74 characterised in that the particle is a nanoparticle.

83. The use according to claims 73 or 74 characterised in that the method comprises the remote manipulation of a cell.

84. The use according to claims 73 or 74 characterised in that the cell is a mammalian cell.

85. The use according to claims 73 or 74 characterised in that the cell is a bacterial cell.

86. The use according to claims 73 or 74 characterised in that the cell is a plant cell.

87. The use according to claim 83 characterised in that the cell is derived from connective or neuronal tissue.

88. The use according to claim 87 characterised in that the cell is derived from bone, neurons, cardiac cells or any combination thereof.

89. The use according to claim 74 characterised in that the ion channel is a mechanosensitive ion channel.

90. The use according to claim 89 characterised in that the mechanosensitive ion channel has been transfected into a cell.

91. The use according to claims 89 or 90 characterised in that the ion channel is a voltage-gated ion channel.

92. The use according to claims 66 or 67 characterised in that the ion channel is a ligand-gated ion channel.

93. The use according to claim 74 characterised in that the ion channel is selected from the group a including sodium channel, potassium channel, calcium channel, chloride channel and a non-selective cation channel or any combination thereof.

94. The use according to claim 93 characterised in that the ion channel is selected from a calcium or a potassium ion channel.

95. The use according to claim 94 characterised in that the ion channel is a potassium ion channel.

96. The use according to claim 94 characterised in that the potassium channel is a TREK-1 channel.

97. The use of a magnetisable particle in the manufacture of a system for use in a method of manipulating a mechanosensitive ion channel characterised in that the method comprises the association of a magnetisable particle with an ion channel.

98. The use according to claim 73, 74 or 97 characterised in that the magnetisable material is selected from the group, which includes elemental iron (Fe), or a compound thereof and a chromium compound, or a combination thereof.

99. The use according to claim 98 characterised in that the iron compound is an iron salt.

100. The use according to claim 100 characterised in that the iron salt is selected from the group, which includes magnetite (Fe_3O_4), maghemite (γFe_2O_3) and greigite (Fe_3S_4), or any combination thereof.

101. The use according to claim 98 characterised in that the chromium compound is a chromium salt.

102. The use according to claim 101 characterised in that the chromium salt is chromium oxide (CrO_2).

103. The use according to claim 73, 74 or 97 characterised in that the magnetisable material comprises particles which comprise a magnetic core with a biocompatible coating.

104. The use according to claim 103 characterised in that the particle has a core and a silica shell enveloping the core.

105. The use according to claim 104 characterised in that the particle is selected from those comprising (a) a core comprising a magnetisable particle and (b) a silica shell enveloping the core.

106. The use according to claim 105 characterised in that the magnetisable particle is selected from the group, which includes elemental iron (Fe), or a salt thereof and a chromium salt, or a combination thereof.

107. The use of a magnetisable particle in the manufacture of a system for inducing a therapeutic effect in a cell which comprises agonising or antagonising ion channels within the cell by the association of a magnetisable particle with the cell and magnetically manipulating the magnetisable particle.

108. The use according to claim 107 characterised in that a therapeutically active agent is administered simultaneously, separately or sequentially with agonising or antagonising ion channels within the cell.

109. The use of a magnetisable particle in the manufacture of a system for targeting a therapeutically active agent to a cell which comprises agonising or antagonising ion channels within the cell by the association of a magnetisable particle with the cell, magnetically manipulating the magnetisable particle and simultaneously, separately or sequentially administering the therapeutically active agent.

110. The use according to claim 97 characterised in that the particle is a porous particle with multiple magnetic centre within the pores.

111. The use according to claim 73, 74 or 97 characterised in that the particles have a mean size of 5000 nm or less.

112. The use according to claim 110 characterised in that the particles have a mean size of from 1 nm to 5000 nm.

113. The use according to claim 73, 84 or 97 characterised in that the method comprises the application of a remote magnetic field on the magnetisable particles.

114. The use according to claim 73, 74 or 97 characterised in that the particle is tagged with one or more specific antibodies or protein binding motifs which recognise key cellular elements within a cell.

115. The use according to claim 114 characterised in that the specific antibodies or protein binding motifs are selected from transmembrane extracellular matrix molecules, adhesion molecules or dispersed membrane adhesion proteins or extracellular matrix proteins.

116. The use according to claim 115 characterised in that the method is *in vivo*.

117. The use according to claim 115 characterised in that the specific antibodies or protein binding motifs are transmembrane adhesion or extracellular matrix molecules.

118. The use according to claim 117 characterised in that the transmembrane adhesion molecules are selected from integrins, cadherins, selectins, and immunoglobulins.

119. The use according to claim 116 characterised in that the specific antibodies or protein binding motifs are selected from dispersed membrane adhesion proteins.

120. The use according to claim 119 characterised in that the dispersed membrane adhesion protein is RGD (arginine-glycine-aspartate).

121. The use of a magnetisable particle in the manufacture of a system for the treatment of a patient suffering from a disorder in which an ion channel plays a role which comprises the administration to such a patient of magnetisable particles as hereinbefore described and manipulating the ion channels or cells using a magnetic field external to the body.

122. The use of a magnetisable particle in the manufacture of a system for destroying cells or inhibiting cell growth which comprises agonising or antagonising ion channels within a cell by the association of a magnetisable particle with a cell.

123. The use of a magnetisable particle in the manufacture of a system for inducing osmotic shock to a cell which comprises agonising or antagonising ion channels within a cell by the association of a magnetisable particle with a cell.

124. The use of a magnetisable particle in the manufacture of a system for the treatment or alleviation of a tumour cell which comprises a method according to claim 49.

125. The use according to claim 124 characterised in that the tumour cell is a cancer cell.

126. The use according to claim 122 characterised in that the method comprises the killing of cells via holding ion channels open with a targeted static magnetic field.

127. The use according to claim 122 characterised in that the method comprises the killing of cells via cyclically opening and closing ion channels with a targeted, time-varying magnetic field.

128. The use according to claim 121 in which a disorder may involve a number of tissues in the body where ion channels play a key role in normal cellular homeostasis.

129. The use according to claim 128 characterised in the cells are cardiac muscle cells.

130. The use according to claim 128 characterised in that the use comprises the manufacture of a system for the treatment of hypertension.

131. The use according to claim 128 characterised in that the use comprises the manufacture of a system for the treatment of pain relief.

132. The use according to claim 131 characterised in that the use comprises the manufacture of a system for the treatment of anaesthesia.

133. The use according to claim 132 characterised in that the anaesthesia is localised.

134. The use according to claim 121 characterised in that the use comprises the manufacture of a system for tissue and/or bone repair.

135. The use of treatment according to claim 134 characterised in that the cells are selected from ligamentum cells, tenocytes, chondrocytes and other stromal cells (such as chondrocyte progenitor cells).

136. The use according to claim 134 characterised in that the use comprises the manufacture of a system for the regeneration of tissue or the generation of artificial tissue, such as skin, cartilage, ligament, tendon, muscle or bone.

137. The use according to claim 134 characterised in that the system utilises remote activation of ion channels.

138. The use according to claim 134 characterised in that the use comprises the manufacture of a system for wound healing and/or tissue adhesion.

139. The use according to claim 134 characterised in that the use comprises the manufacture of a system for bone repair and/or bone growth.

140. The use according to claim 121 characterised in that the use comprises the manufacture of a system for dental or veterinary application.

141. The use of a magnetisable particle in the manufacture of a system for establishing localised anaesthesia through the action of ion channel modulation by a magnetic field external to the body.

142. The use according to claim 141 characterised in that the pain relief comprises anaesthesia.

143. The use according to claim 121 characterised in that the method comprises the use of a magnetic field at a frequency of from 0.1 to 10 Hz.

144. The use according to claim 121 characterised in that the method comprises the use of a magnetic field will typically have a flux density of from 10 mT to 1400 mT.

145. The use according to claim 116 characterised in that the use comprises manipulating cells from outside the body.

146. The use according to claim 116 characterised in that the use comprises a method of tissue repair and/or bone repair.

147. The use according to claim 116 characterised in that the use comprises a method of pain relief.

148. The use according to claim 147 characterised in that the pain relief comprises anaesthesia.

149. The use of a magnetisable particle in the manufacture of a system for inducing a therapeutic effect in a cell which comprises agonising or antagonising ion channels within the cell by the association of a magnetisable particle with the cell and magnetically manipulating the magnetisable particle.

150. The use of a magnetisable particle in the manufacture of a system comprising a therapeutically active agent which may be administered simultaneously, separately or sequentially with the magnetisable particle whilst agonising or antagonising ion channels within the cell.

151. The use of a magnetisable particle in the manufacture of a system for targeting a therapeutically active agent to a cell which comprises agonising or

antagonising ion channels within the cell by the association of a magnetisable particle with the cell, magnetically manipulating the magnetisable particle and simultaneously, separately or sequentially administering the therapeutically active agent.

152. A kit comprising a therapeutically active agent and means for associating a magnetisable particle with a cell.

153. A method or use substantially as described with reference to the accompanying drawings.

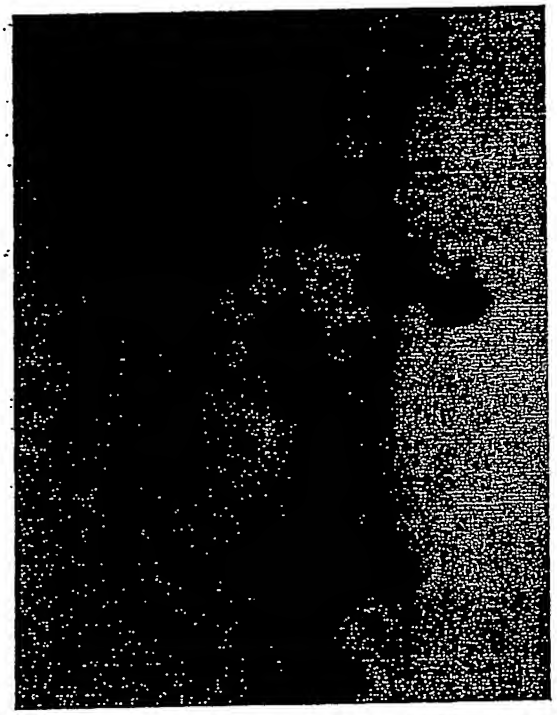


Figure 1b

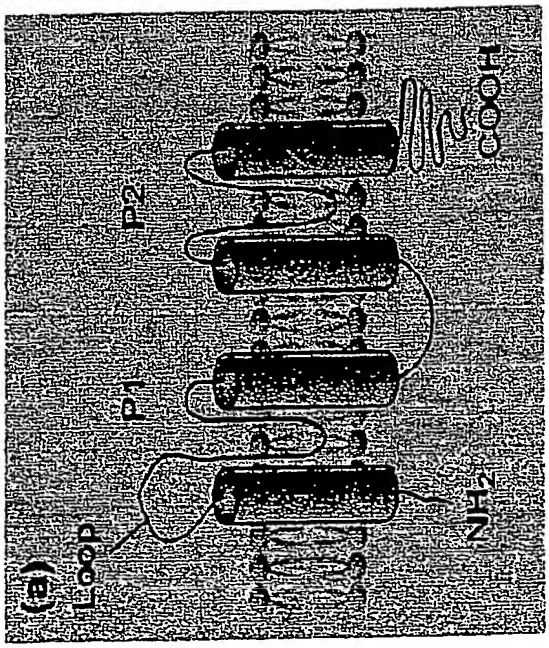


Figure 1a

TREK-1 channel structure

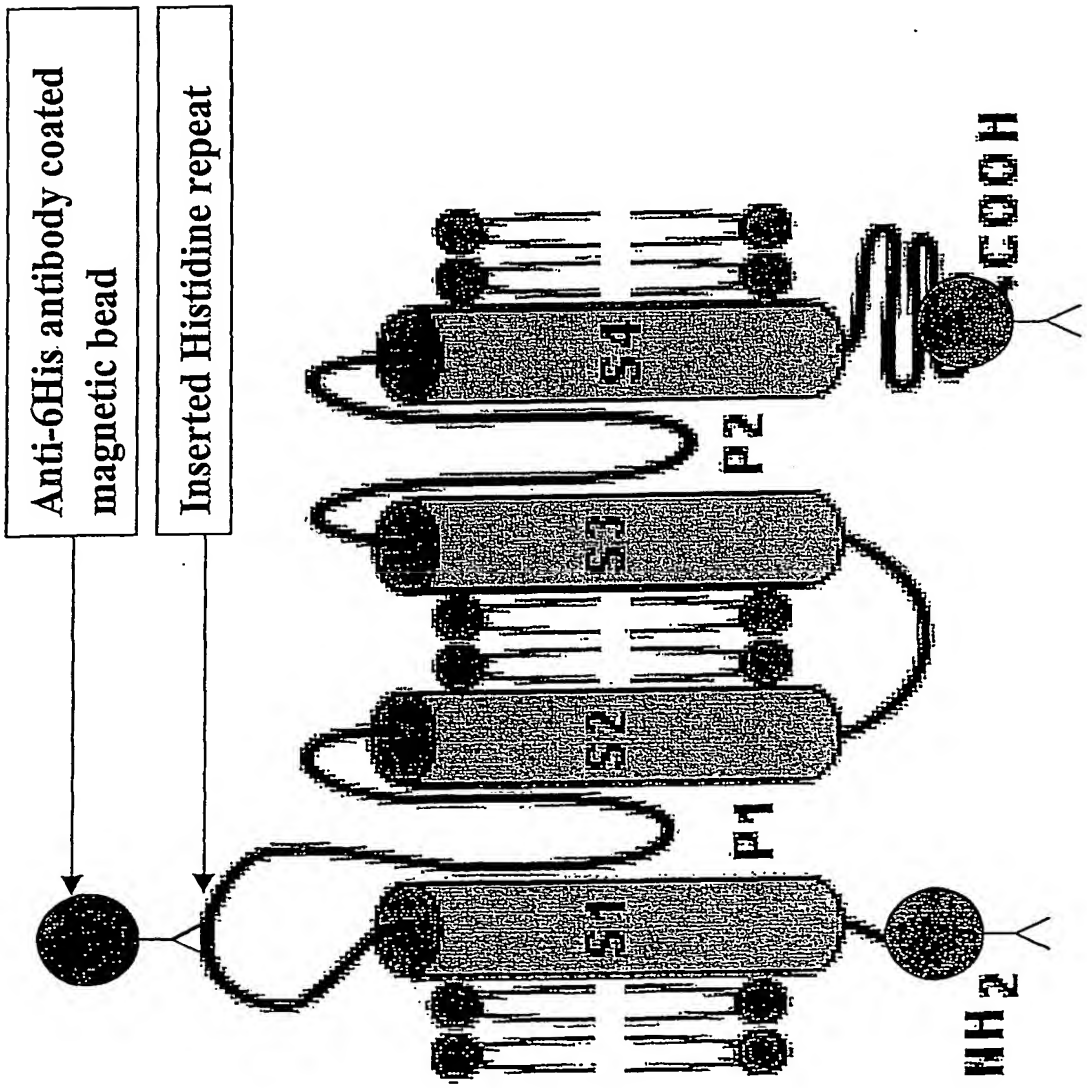


Figure 2 : Magnetic activation of Trek-1 monitored via downstream changes in intracellular calcium

• COS-7 cells cotransfected with 'Flash' pericam and 12His.Trek-1 – Red and yellow lines indicate the cells with magnetic particles attached. Green and White lines indicate the cells without magnetic particles attached.

•Magnetic field applied for 1 sec – yellow arrow.

•Application of 100uM Riluzole to the culture media – red arrow

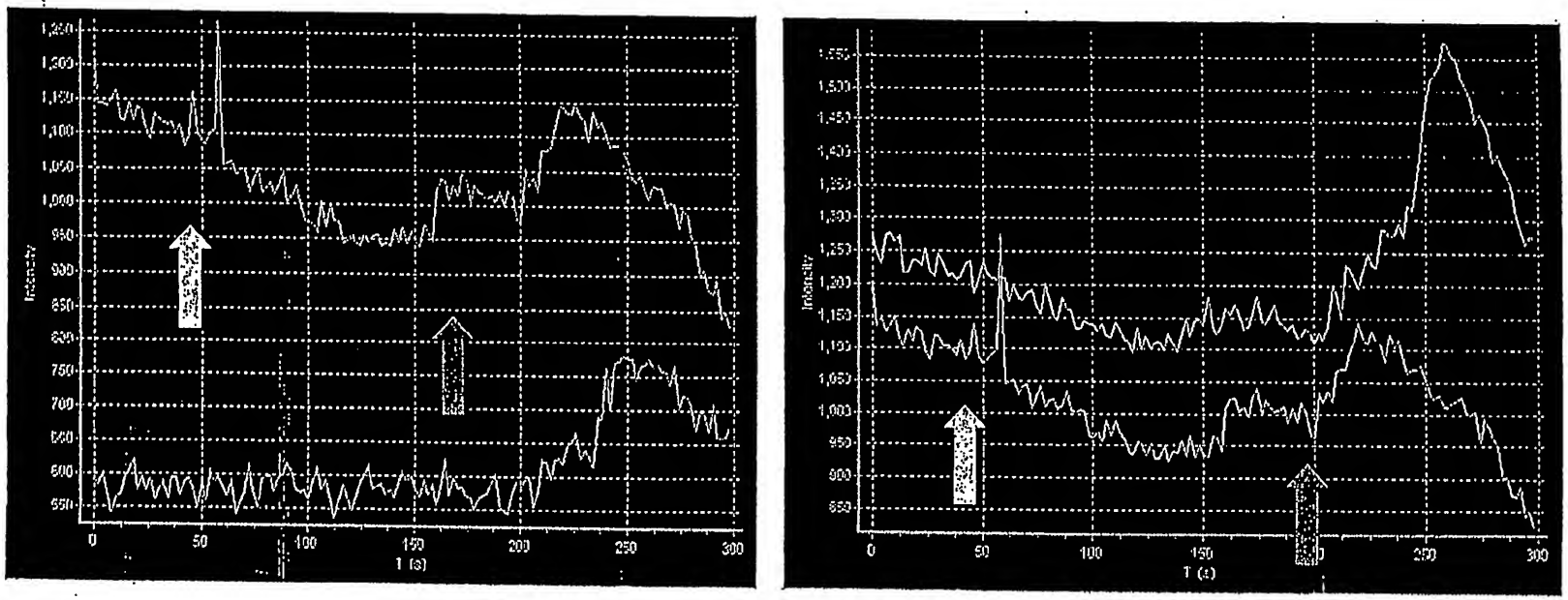
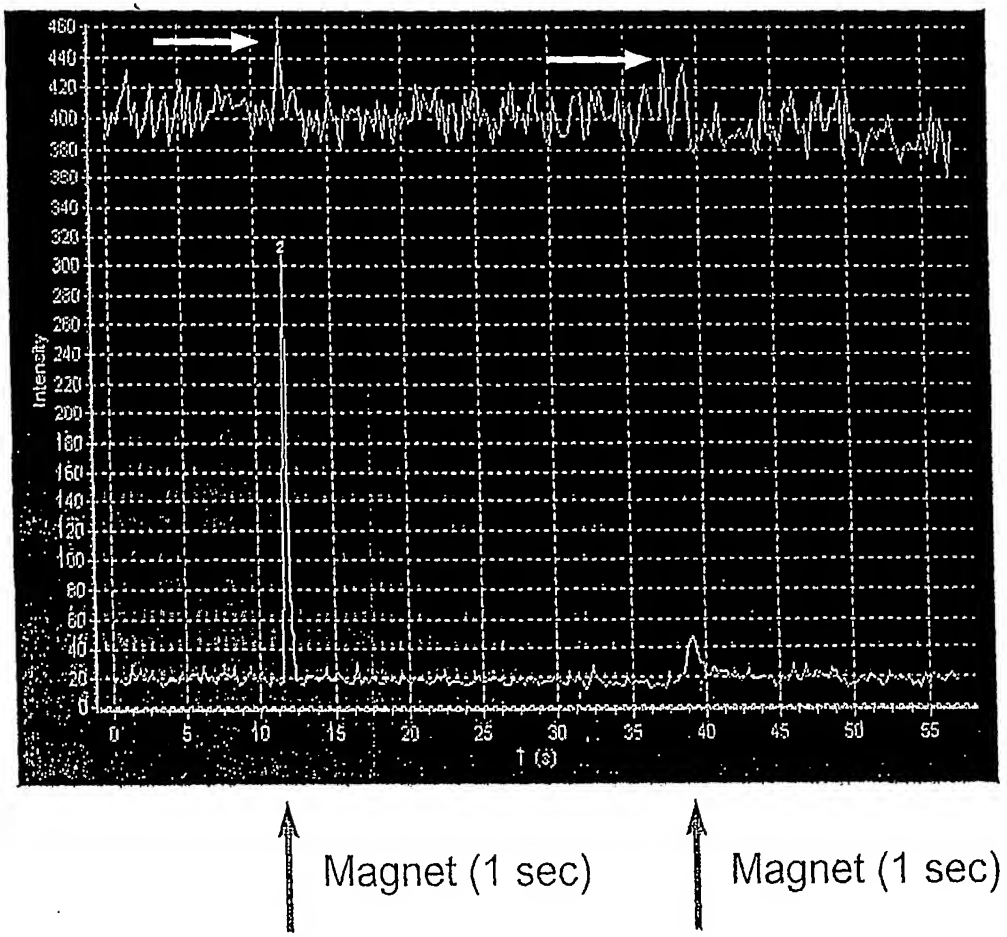


Figure 3 : Magnetic activation of TREK-1 induces transient rise in intracellular calcium in HEK293 T cells co-transfected with and Flashpericam



Channel 1= Green = HEK293 cells with magnetic particle attached = white arrow indicates calcium response

Channel 2 = demonstrates movement of the particles attached to the cells following application of the magnet (indicated by a red arrow)